THE KISSPEPTIN SYSTEM: A NOVEL REGULATOR OF THE REPRODUCTIVE AXIS IN TELEOST FISHES

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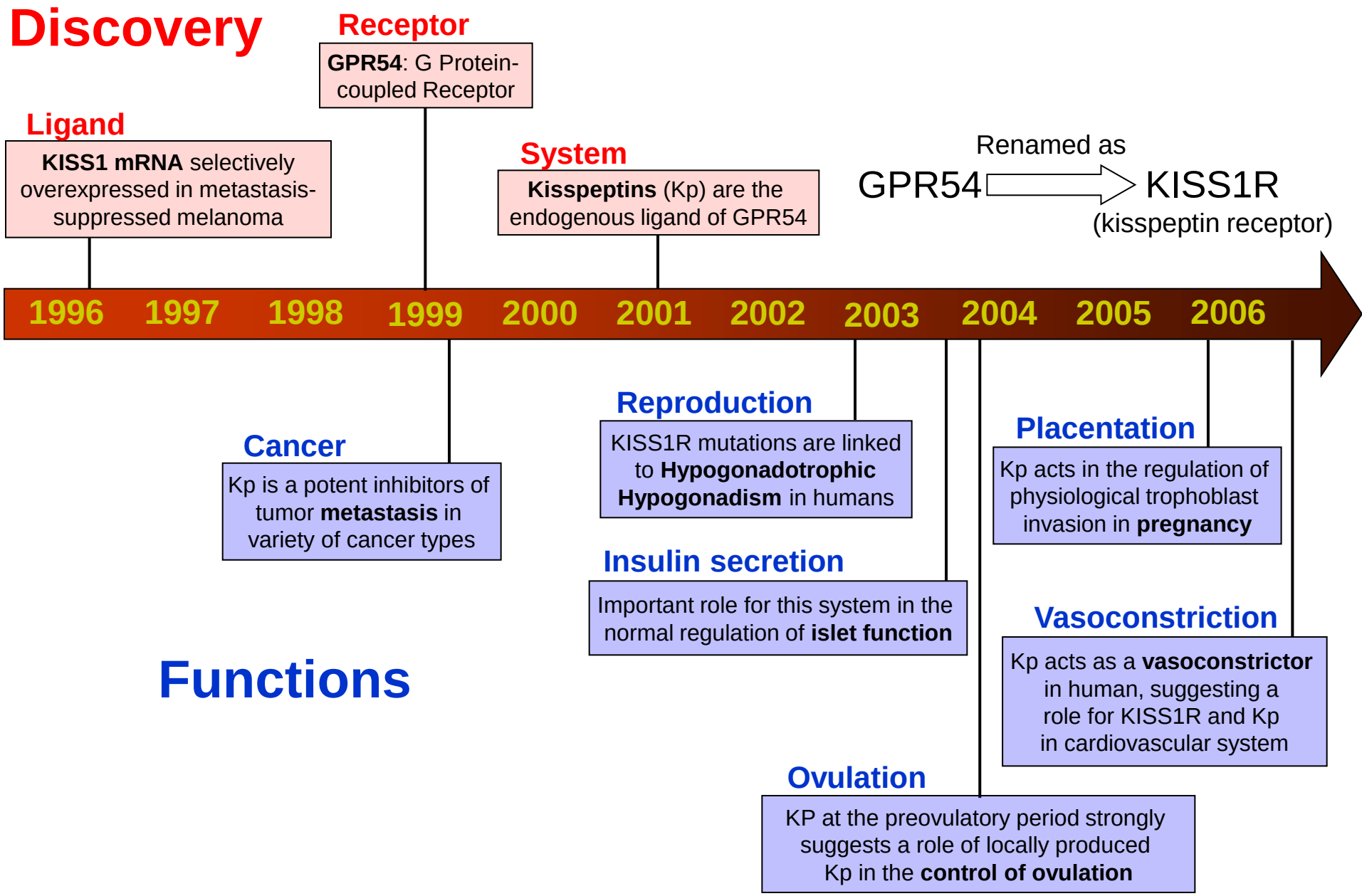
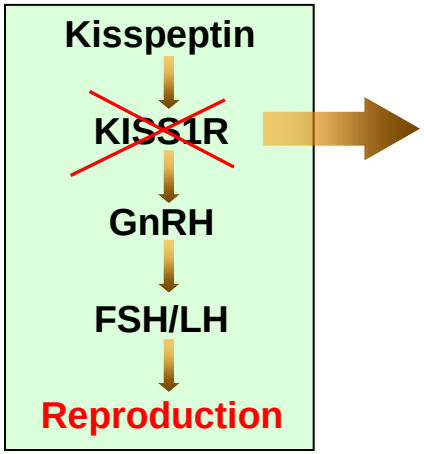
Kisspeptin system. Discovery of elements and functions

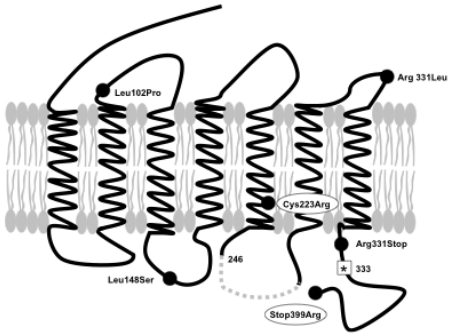


Kisspeptin system role in Reproduction (puberty)

de Roux, Funes and Seminara

In 2003, three groups identified the role of the KISS1R in **reproduction**



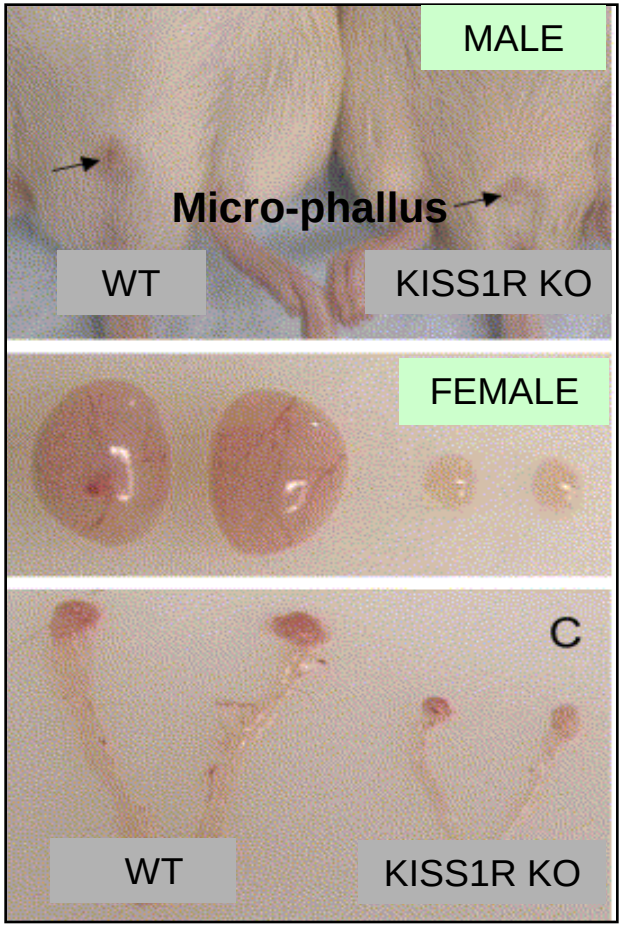


Roa et al., 2008

When **KISS1R is mutated**, mammals **do not undergo puberty**, their gonads are small, their sex hormones and gonadotropin levels are low, and they are sterile. **Hypogonadotropic hypogonadism.**

Messager, 2004; de Roux et al, 2003

Comparison of Wild-type (WT) and KISS1R knock out (KO) reproductive phenotypes



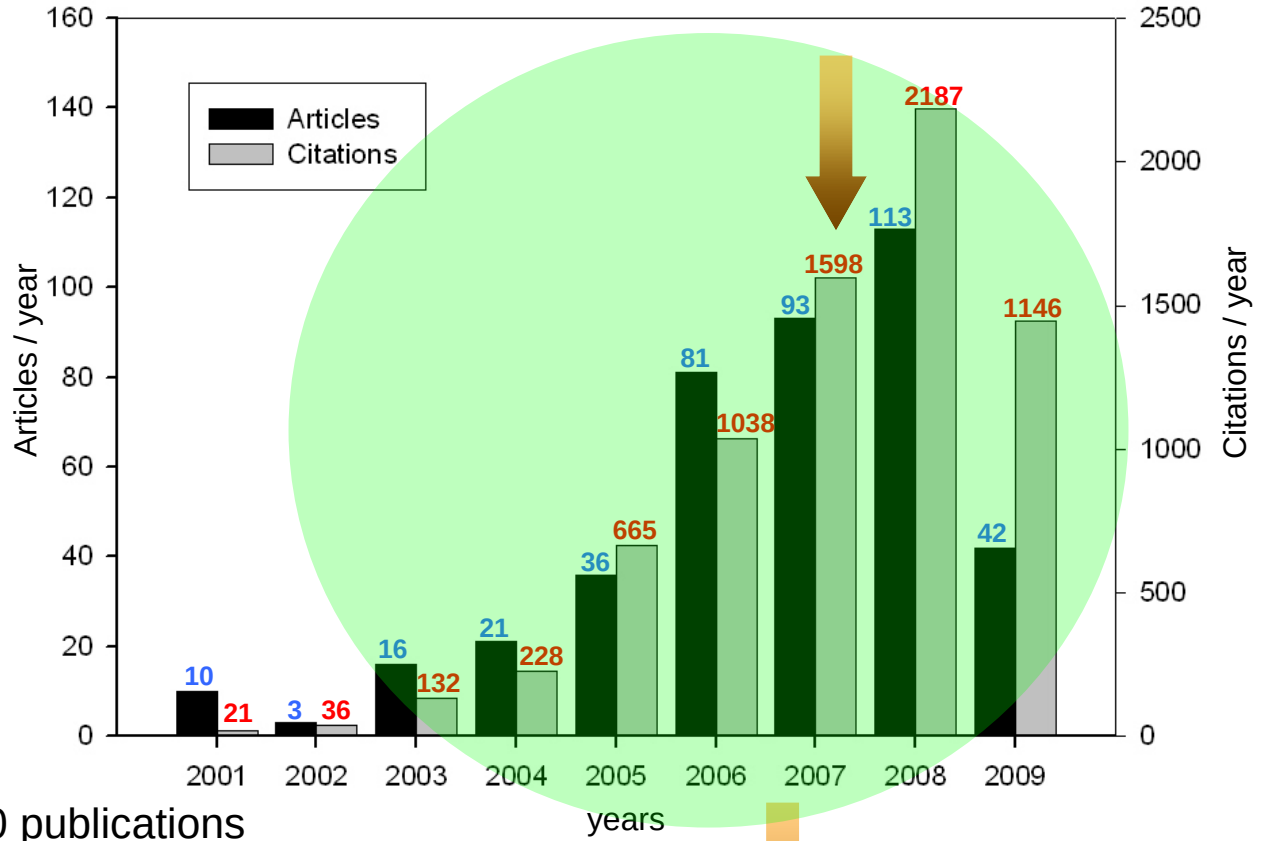
Funes et al., 2003

Kisspeptin system. Relevance in reproduction

ISI search
(Using 5 keywords)

METASTIN
KISSPEPTIN
KISS1R
GPR54
KISS1

Total articles = 409



In 2007, 5 of the top10 publications in biology of reproduction

2008 Nature Medicine. Vol. 14 num 11

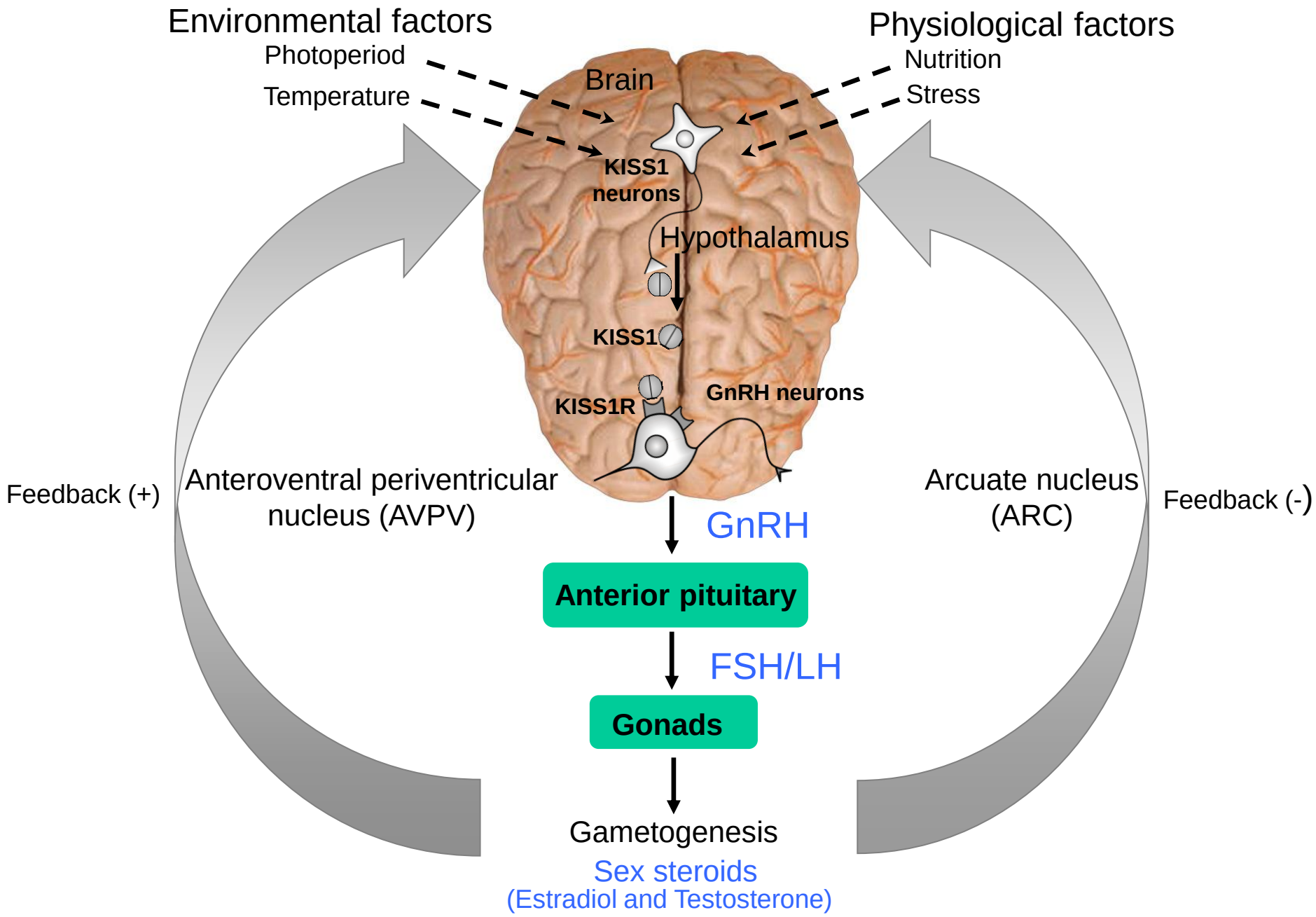
54 % of the total publications

In the last 5 years, the **kisspeptin system** has emerged as a **critical regulator of reproduction**

"The discovery of the role of **KISSPEPTIN** and its receptor, **KISS1R**, in **PUBERTY** is the most exciting finding made in the field of reproductive biology since the discovery of GnRH in the 1970s"

Messager, 2005. J. Neuroendocrinol. Vol. 17, 10: 687-688

Regulation in the Hypothalamic-pituitary-gonadal axis (HPG)



Rationale

Flatfish aquaculture

Understanding of the control of the initiation of puberty in fish is a basic attention in the aquaculture industry

Problems in reproduction of Flatfish aquaculture:

Solea senegalensis

Delay of puberty and failure of reproduction in F1 males

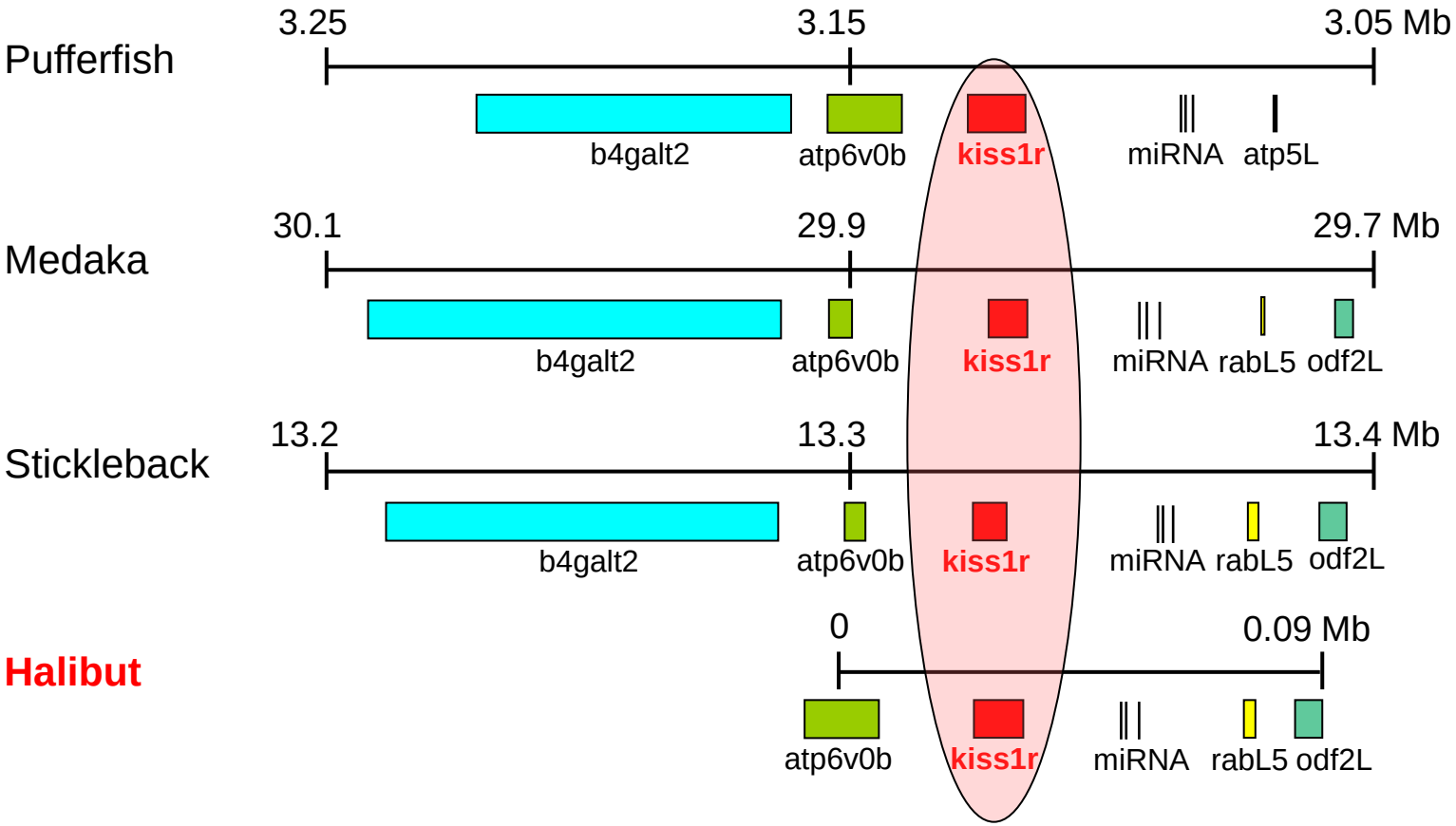
Atlantic halibut

Early maturation and reduced growth rates of males

"Basic studies concerning the control of reproduction are needed"

Objective of this study:
"Characterization the kisspeptin system in flatfish"

KISS1R synteny in teleost fishes



Mechaly et al., 2009 Mol. Cel. Endocrinol. Submitted

The highly conserved nature of the kiss1r region suggests that the flanking regulatory sequences are conserved

KISS1R promoter in teleost fishes

Atlantic halibut

Genomic library screening

Promoter

? ? ?

5´UTR ATG Exon I I Exon II II Exon III III Exon IV IV Exon V 3´UTR

Translation

Transcription

Mechaly et al., 2009 (Submitted)

Bioinformatics

KISS1R promoter in teleost fishes

Putative transcription factors binding sites

-1000 -800 -600 -400 -200 0

Medaka

Stickleback

Pufferfish

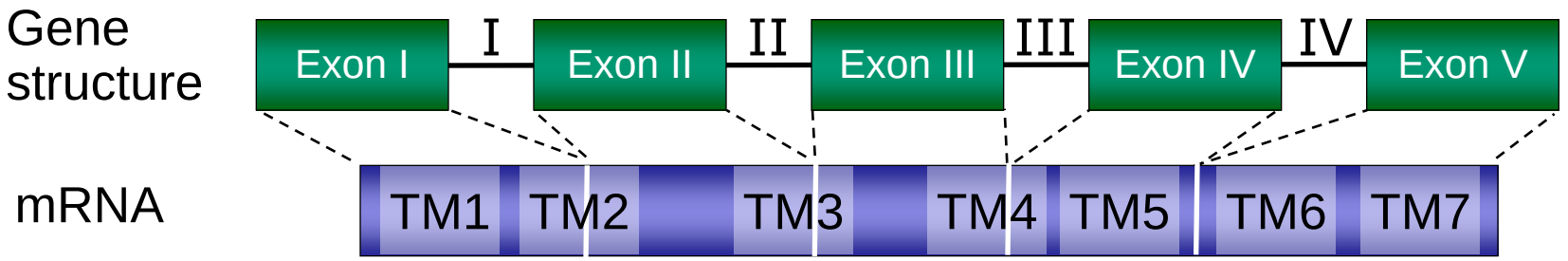
Halibut

MIF-1 binding site

Estrogen receptor binding site

Two regions showed considerable similarity among species of the localization of the putative binding sites for the transcription factors MIF-1 and estrogen receptor

Alternative splicing of KISS1R in vertebrates



alternative splicing mechanisms

INTRON RETENTION

First alternative spliced isoform detected in KISS1R in any species

Mechaly et al., 2009 Biol. Reprod. 80: 60-69

Senegalese sole

insertion 81bp

TM1 TM2 TM3 TM4 TM5 TM6 TM7

Sheep

insertion 105bp

TM1 TM2 TM3 TM4 TM5 TM6 TM7

In silico analysis

TRANSMEMBRANE DOMAIN DELETION

Non- functional isoform ?

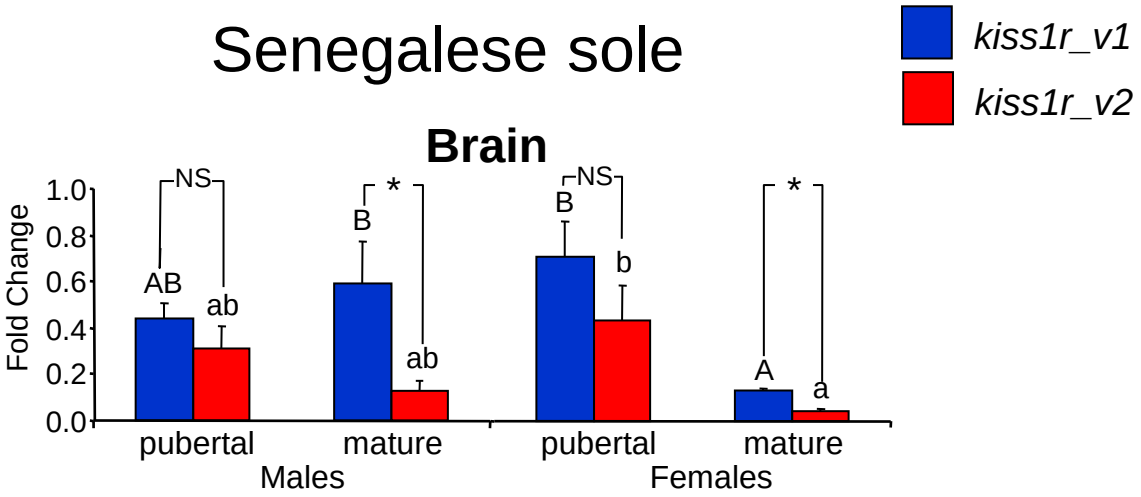
Mouse

deletion TM5

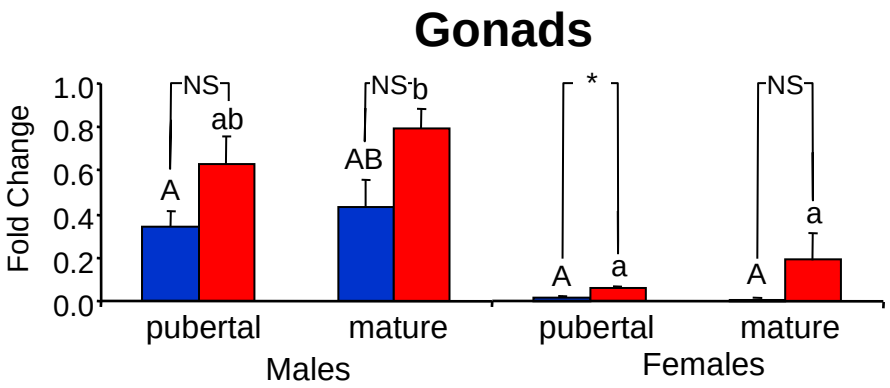
TM1 TM2 TM3 TM4 TM6 TM7

KISS1R expression in brain and gonads of flatfishes

Senegalese sole

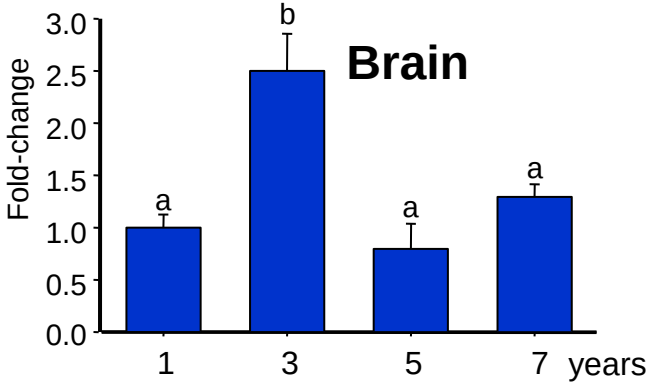


Higher expression of Solea *kiss1r_v1*

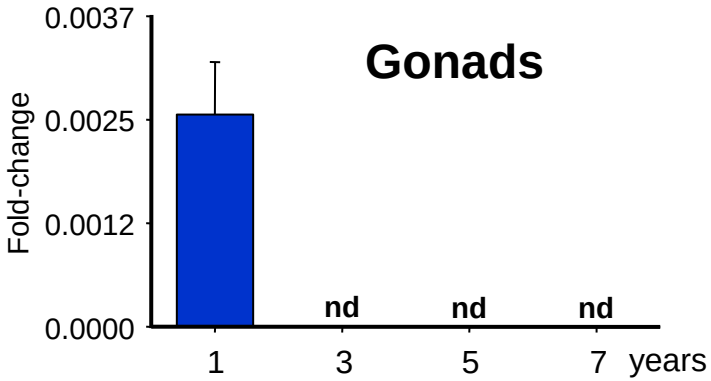


Higher expression of Solea *kiss1r_v2*

Atlantic halibut



Higher expression of Halibut *kiss1r* in the onset of puberty



Two orders of magnitude lower than brain

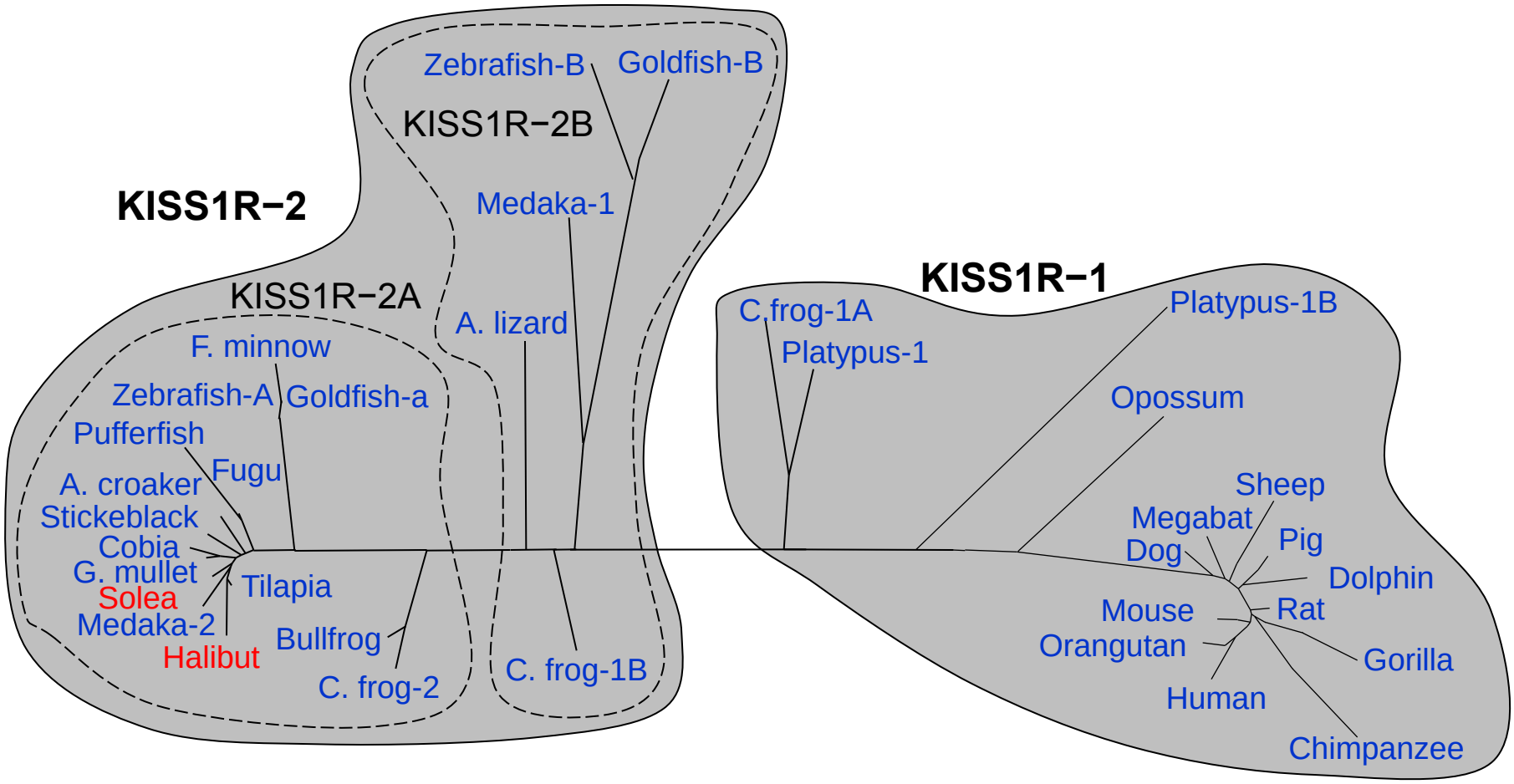
Differential expression patterns of the two different transcripts in brain and gonads

Mechaly et al., 2009 Biol. Reprod. 80: 60-69

Kiss1r presented higher expression in the onset of puberty in brain and only detected in immature fish gonads

Mechaly et al., 2009 (Submitted)

Phylogenetic analysis and gene duplication events of KISS1R



Mechaly et al., 2009 (Submitted)

Phylogenetic analysis grouped the Kiss1r sequences into three groups: *Kiss1-r1*, *Kiss1r-2A* and *Kiss1r-2B*, and revealed some unexpected relationships, showing that, in contrats to what had been surmised earlier, Kiss1r-2B is a non-mammalian form of Kiss1r.

KISS1R expression in brain of vertebrates (Summary of results)

Species	Gene type	Pre-pubertal	Pubertal			Post-pubertal
		Inmature	Onset	Medium	Advance	Mature
Mammals						
Human		+	+++			
Rat		+		+++		
Teleost fishes						
Nile tilapia		++				+++
Fathead minnow		+	+++	++	+	+
Cobia	*Kiss1r2*	++	+++			
Grey Mullet	*Kiss1r2*		+++	++	+	
Zebrafish	*kiss1ra*	+	+	+	+++	+
	kiss1rb	+	+	+++	+++	+++
Senegalese sole	*Kiss1r_v1*		++			+
	Kiss1r_v2		++			+
Atlantic halibut	*Kiss1r-2*	+	+++		+	+

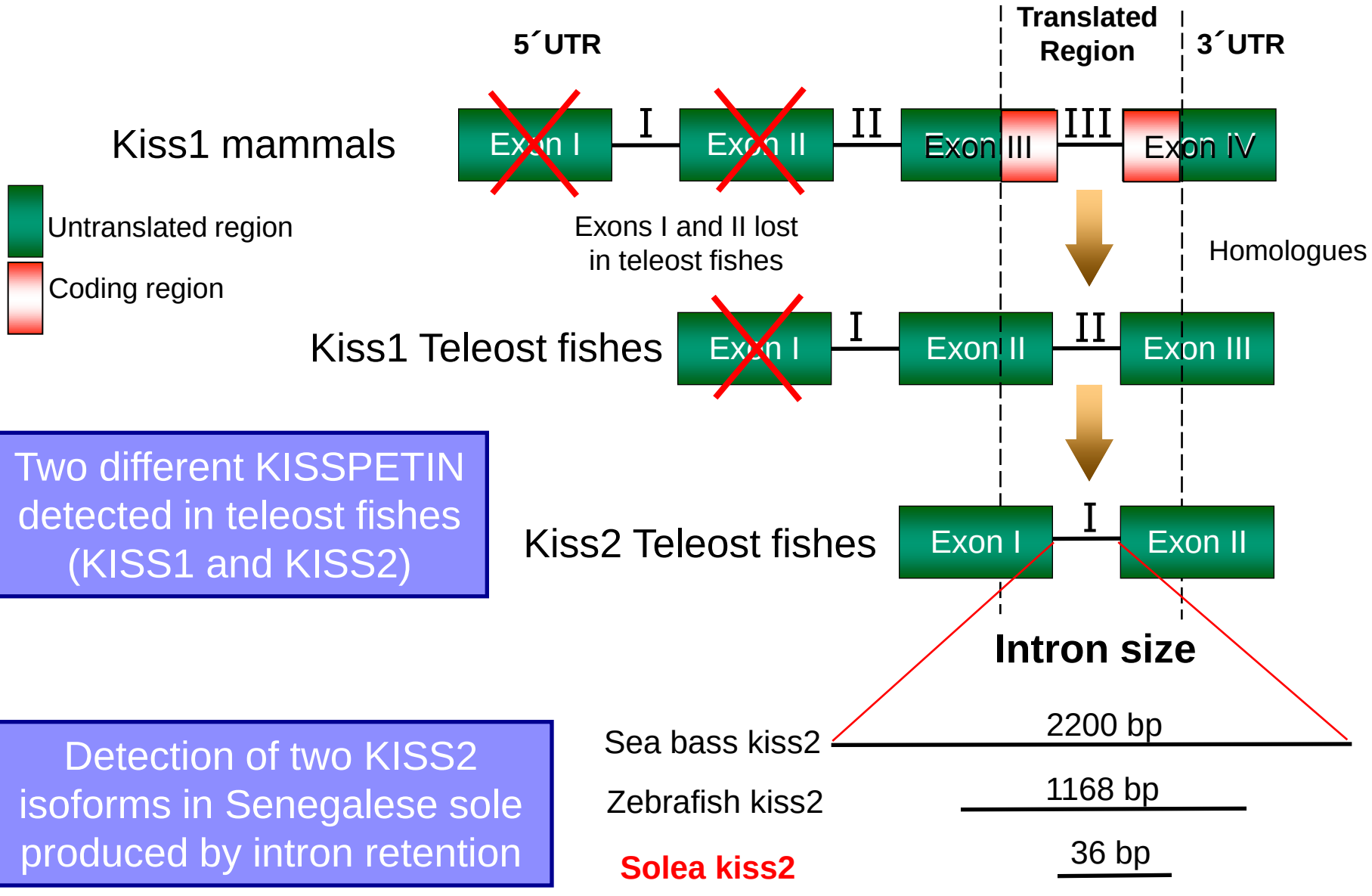
Mechaly et al., 2008 Treb. Soc. Cat. Biol. 59: 95-107

Differences in KISS1R expression between pubertal and mature and in different genes

No consistent results

Multiple genes or alternative splicing undetected events in other species ?

Kisspeptin gene structure in vertebrates



Two different KISSPETIN detected in teleost fishes (KISS1 and KISS2)

Detection of two KISS2 isoforms in Senegalese sole produced by intron retention

Mechaly, Viñas and Piferrer. Unpublished

Specific conclusions

1- Synteny analysis showed a highly conserved nature of the *kiss1r-2* region, suggesting that flanking regulatory sequences are also likely to be conserved.

2- Bioinformatic analysis identified for the first time six conserved regions in piscine *kiss1r-2* upstream sequences, providing potential targets for *kiss1r-2* regulation in different species.

3- We found two different isoforms of *Kiss1r* generated by alternative splicing, originated by the retention of intron 3.

4- In the brain of Senegalese sole and *Atlantic halibut kiss1r_v1* was the most abundant isoform during the onset of puberty.

5- In the gonads, of Senegalese sole, *kiss1r_v2* was the most abundant with lower levels in females than in males. In Atlantic halibut, kiss1r expression levels were lower (2 order of magnitude) than those of the brain and were detected only in immature fish.

6- The kiss1r-2B is a non-mammalian form of kiss1r. *Kiss1r-1* was not found in sole and halibut, indicating that in the most derived teleosts, including the Pleuronectiformes and Tetraodontiformes, this gene may been lost.

General conclusions

1- The several functions associated with the kisspeptin and its receptor, KISS1R, confirm that this is a pleiotropic system

2- The different genes and isoforms, originated by duplication and alternative splicing, respectively, found in different species, add to the complexity of the kisspeptin system

3- The relative conservation of this system across vertebrates demonstrates their importance in the regulation of essential biological processes.


Moltes gràcies